

Estimation of Effect of Environmental Tobacco Smoke on Air Quality within Passenger Cabins of Commercial Aircraft

Guy B. Oldaker III* and Fred C. Conrad, Jr.

Research and Development Department, Bowman Gray Technical Center, R. J. Reynolds Tobacco Company, Winston-Salem, North Carolina 27102

Nicotine was measured in passenger cabins of Boeing B727-200, B737-200, and B737-300 aircraft in order to estimate the levels of environmental tobacco smoke (ETS) and to assess the effectiveness of smoker segregation as a means of reducing nonsmokers' exposure to ETS. Integrated sampling was performed at seats in smoking and no-smoking sections on flights averaging 55 min. Nicotine was collected on XAD-4 resin and analyzed by gas chromatography with nitrogen-phosphorus detection. Results indicate that significant nicotine concentration gradients exist in cabins and that concentrations increase in magnitude from no-smoking sections to smoking sections. The mean nicotine concentration for samples acquired in no-smoking sections was $5.5 \mu\text{g}/\text{m}^3$; in smoking sections of aircraft the mean nicotine concentration was $9.2 \mu\text{g}/\text{m}^3$. These concentrations correspond to estimated mean exposures of 0.0041 and 0.0082 cigarette equivalent per flight, respectively.

Introduction

In the U.S., commercial airlines are required during flights to segregate smokers in order to reduce the exposure of nonsmokers to environmental tobacco smoke (ETS), defined as the mixture of diluted and aged sidestream smoke and exhaled mainstream smoke. Since the implementation of this requirement (1), its consequences for cabin air quality have not been systematically studied. Although data relative to the levels of ETS in aircraft are contained in a report (2) issued jointly by the U.S. Department of Health, Education and Welfare (DHEW) and the U.S. Department of Transportation (DOT), they were obtained before segregation was required.

The literature contains only one report dealing with the quantitation of ETS levels in passenger cabins. Muramatsu et al. (3) reported the results of seven samples of vapor-phase nicotine collected during Japanese domestic flights. These researchers, however, provided no information on sampling locations. The choice of nicotine as an indicator of ETS reflects the fact that this compound is uniquely specific for tobacco smoke. At the time these results were reported, the relation between vapor-phase nicotine and ETS had not been characterized. Eudy et al. (4) have since then shown that at least 95% of the nicotine associated with ETS exists in the vapor phase.

For the study reported here, vapor-phase nicotine was sampled in passenger cabins of U.S. domestic aircraft in order to gain additional information regarding ETS levels therein and to assess the effectiveness of smoker segregation as a means of reducing the exposure to ETS by persons seated in no-smoking sections. Samples were collected unobtrusively with systems contained in ordinary briefcases in order not to disturb the behavior of passengers or to disrupt airline operations.

Experimental Section

Sampling System. Samples were acquired with sampling systems contained in briefcases that were carefully designed to be inconspicuous (Figure 1). Brass sample

inlet and exhaust ports and the on-off switch were located on the front of each briefcase and were positioned symmetrically about the handle. Sample ports were fashioned from 0.25-in. o.d. Swagelok port connectors. Tubing extensions of port connectors were removed, and the resulting flat surfaces were polished. In addition, one of the port connectors was drilled out to a diameter of 0.25 in. to accommodate 6-mm o.d. XAD-4 sorbent tubes.

Major components of the system for sampling nicotine included an XAD-4 sorbent tube and a constant-flow sampling pump (both obtained from SKC, Inc., Eighty Four, PA). Each XAD-4 sorbent tube was positioned through the fitting on the briefcase's front so that approximately 3 mm of the tube's tip projected. Sorbent tube outlets were connected to sampling pumps with short lengths of rubber tubing. Sampling pumps were calibrated with a film flow meter, and flow rates were set at 1 L/min. Calibrations were confirmed with a mercury film flow meter. Flow rates were computed at standard conditions: 295 K (25 °C) and 760 Torr. Temperature and pressure data for adjusting calibration results to standard conditions were obtained from a mercury-in-glass thermometer and a mercury-in-glass barometer, respectively. According to protocol, calibrations were checked at weekly intervals throughout the study. Results from sampling were judged acceptable if the calibrations remained within $\pm 5\%$.

Sampling Procedure. A written sampling protocol was prepared in conjunction with the study. Persons conducting the sampling were provided with this protocol and also were orally briefed at the start of the study. In addition, persons conducting the sampling had security clearances that permitted them to pass through security stations without revealing the briefcases' contents. All but 14 of the samples were acquired by airline employees, who agreed to participate in the study gratis. The protocol directed that none of the persons conducting the sampling was to smoke during the times when samples were acquired. All sampling operations were performed during scheduled commercial flights that involved business unrelated to the study. Persons conducting the sampling selected flights strictly on the basis of availability and made no effort to select among aircraft types. None of the aircraft that figured in the study had first-class compartments; each aircraft had one smoking section and one no-smoking section.

Sampling was performed during the times when carry-on items such as briefcases could be unobtrusively removed from beneath seats. These times correspond to the times when smoking is permitted in the passenger cabins. Owing to the airline company's seating policy, most samples were obtained at boundary regions between smoking and no-smoking sections. Boundary regions included the last two rows in no-smoking sections adjacent to smoking sections.

Positioning of briefcases during the sampling depended on whether unoccupied seats were available. According to protocol, if an empty seat existed next to the person conducting the sampling, the briefcase was placed in the empty seat and oriented vertically; otherwise, the briefcase was placed in a horizontal position on the sampler's lap

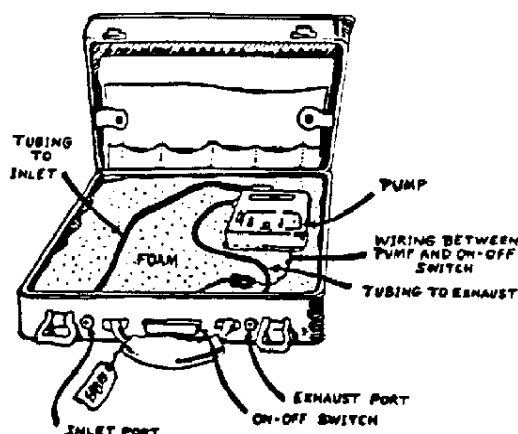


Figure 1. Briefcase sampling system.

with the sampling ports directed away from the body. When briefcases were oriented vertically, samples were acquired within approximately 15 cm of an adult passenger's breathing zone; when briefcases were oriented horizontally, this distance was approximately 45 cm. Airflow to sampling ports was unobstructed. In addition, the protocol specified that the air vents (gaspers) located in the passenger service unit above seats occupied by the briefcase samplers were to be closed during the sample acquisition. The protocol specified that samples be placed in a freezer within 24 h of acquisition.

Barometric pressure was measured on four flights. For the first of these, a hand-held altimeter calibrated against a mercury-in-glass barometer was employed. Response was approximated with a linear least-squares numerical method ($R^2 = 0.999$). Use of the altimeter was determined to be overly conspicuous and burdensome, and consequently its use was discontinued. Additional pressure data were provided by a pressure transducer (Omega Engineering, Inc., Stamford, CT) installed in the briefcase. The transducer was calibrated with a mercury-in-glass barometer and interfaced with a 21X Micrologger (Campbell Scientific, Inc., Logan, UT).

Analytical Procedure. Two methods were used to analyze nicotine, both representing enhancements of the method (5) developed by the National Institute of Occupational Safety and Health (NIOSH). From the beginning of the study to 14 January 1986 (corresponding to sample number 36), samples were analyzed with a Model 5880A gas chromatograph equipped with a nitrogen-phosphorus detector (NPD) and a Model 7672A automatic sampler (Hewlett-Packard, Avondale, PA). The column used was a 30-m DB-WAX fused silica capillary with a 0.32-mm i.d. Injections were performed in splitless mode. Column temperature was programmed from 60 to 210 °C at 12 deg/min. Temperatures for the injector and detector were 250 and 300 °C, respectively. Quantitation was accomplished with the use of quinoline as an internal standard.

The method employed for the remainder of the study entailed the use of a 30-m DB5 megabore column with an internal diameter of 0.53 mm and a film thickness of 1.5 μ m. Temperatures for the injector and detector were 250 and 300 °C, respectively. Column temperature was programmed from 150 to 175 °C at 5 deg/min. In addition, the ethyl acetate solvent was modified to contain 0.01% (v/v) triethylamine.

Chromatographic systems were calibrated at a minimum of five concentration levels for each set of analyses.

Reagent-grade nicotine for these standards was obtained from Eastman Kodak and was used as received. This reagent was stored in a freezer. Field samples were analyzed once; calibration standards were analyzed in duplicate before and after field samples. Results for calibration standards were used in conjunction with a linear least-squares program to compute nicotine levels of field samples and blanks. At least two sample blanks were analyzed with each set of field samples. Nicotine desorption efficiency from XAD-4 resin was determined according to the NIOSH procedure to be 0.92.

Exposures were estimated by computing "cigarette equivalents" from nicotine concentration results and associated sampling times. A breathing rate of 20 L/min (6), corresponding to light activity, was assumed for these calculations. Also assumed was a 1983 sales-weighted average cigarette delivering 0.93 mg of nicotine (7) as measured by the Federal Trade Commission (FTC) method (8, 9).

Results and Discussion

Results of measurements performed in no-smoking and smoking sections of B727-200, B737-200, and B737-300 aircraft are shown in Tables I and II, respectively. These Boeing aircraft types differ among themselves in terms of seating capacity, location of boundary between smoking and no-smoking sections, and operation and design of heating, ventilating, and air conditioning (HVAC) systems. Ventilation systems of B727-200 and B737-200 aircraft are "once-through" systems; i.e., they are incapable of recirculating air within the cabins. Ventilation systems of B737-300 aircraft, on the other hand, recirculate approximately 40% of the air in the passenger cabins (10). Recirculated air is passed through a prefilter and then through a hospital-grade filter (95% efficient for 0.3- μ m particles) to remove particulate matter. The population of aircraft studied may be considered representative inasmuch as modern commercial aircraft utilize both ventilation conditions and the three Boeing aircraft types constitute approximately 50% of the U.S. domestic, commercial aircraft fleet (11).

Seat entries in the tables identify sampling locations. Numbers indicate seating rows, which are numbered from front to back of the aircraft. Accompanying letters designate positions in rows, which for all rows sampled contained six seats, three seats on each side of the aisle. For the aircraft studied, the location of the smoking boundary is variable, depending for each flight on aircraft type, flight demographics, and number of passengers requesting to sit in either of the sections.

The tabulated number of passengers seated in the smoking sections provides an upper estimate of the number of smokers on a particular flight and allows estimation of an upper limit for the number of cigarettes smoked. The number of cigarettes smoked during a flight was estimated by assuming a smoking rate of two cigarettes per hour per passenger seated in the smoking section. This rate is one of two contained in the joint DHEW/DOT report (2); the other measured rate is 0.9 cigarette per smoking passenger per hour. Halpenny and Starrett (12), providing the only other estimate, report 1.34 cigarettes per smoking passenger per hour.

The number of passengers seated in the smoking section was quantified for only a portion of the study. This aspect of the data acquisition process reflected the fact that the study was implemented in phases in order to ensure the quality of results; thus, each successive phase of the study was implemented when data-reliability objectives were met.

Table I. Results from Samples Collected in No-Smoking Sections of B727-200, B737-200, and B737-300 Aircraft

sample	aircraft type	seat	no. in smoking section	no. of cig smoked (std)	sampling time, min	nicotine		cig equiv
						μg	$\mu\text{g}/\text{m}^3$	
85	737-200	1F*	15	20	41	ND (0.02)	ND (0.5)	NA
1	727-200	3D*	20	49	73	ND (0.02)	ND (0.03)*	NA
40	737-200	16C	20	30	45	ND (0.02)	ND (0.04)	NA
64	737-300	16E	5	8	50	ND (0.02)	ND (0.4)	NA
66	737-200	19B	25	76	65	ND (0.02)	ND (0.03)	NA
80	727-200	19B	12	26	65	0.04	0.6	0.0008
83	737-300	2B*	12	17	42	0.09	0.8	0.0007
41	737-200	12C	30	45	45	0.04	0.8	0.0008
26	737-300	15D	NA	NA	50	0.10	1.5	0.0016
82	737-200	11E	NA	NA	66	0.24	1.6	0.0022
69	737-200	9A*	25	63	76	0.17	1.7	0.0027
46	737-200	15C	1	1	41	0.08	1.8	0.0016
3	727-200	19F	20	49	73	0.14	1.9*	0.0029
8	737-300	4F*	NA	NA	39	0.10	2.1	0.0018
2	727-200	19E	20	49	73	0.17	2.3*	0.0037
72	737-200	NA	25	44	53	0.15	2.3	0.0027
32	727-200	19C	NA	NA	40	0.11	2.4	0.0021
75	737-300	6B*	NA	NA	32	0.11	2.7	0.0018
7	727-200	22C	NA	NA	132	0.44	2.7	0.0077
21	737-200	14D	NA	NA	61	0.27	3.3	0.0036
44	727-200	20C	14	16	34	0.13	3.4	0.0025
39	737-300	19C	7	13	57	0.28	4.3	0.0052
77	737-200	12B*	6	7	36	0.21	4.4	0.0034
31	737-200	15E	NA	NA	30	0.20	6.4	0.0041
33	737-200	15D	NA	NA	45	0.33	6.4	0.0062
17	737-200	15D	NA	NA	42	0.39	6.8	0.0062
20	737-200	16C	NA	NA	76	0.88	7.2	0.0117
10	737-300	11D*	NA	NA	39	0.40	8.1	0.0068
29	737-200	15D	20	30	45	0.47	10.0	0.0097
13	737-200	11C*	NA	NA	20	0.27	10.1	0.0044
19	737-200	15D	NA	NA	20	0.27	10.1	0.0044
38	737-200	15C	7	12	50	0.64	11.2	0.0120
81	737-300	15E	20	11	17	0.45	11.7	0.0043
80	737-200	15A	15	56	111	2.76	12.8	0.0306
71	737-200	16B	6	8	41	0.71	14.3	0.0126
70	727-200	20E	40	88	66	1.36	14.6	0.0207
30	737-200	15B	25	29	35	0.53	14.6	0.0110
64	737-200	16B	8	15	55	0.89	15.4	0.0182
73	737-200	15C	30	48	48	0.95	16.6	0.0172
58	737-300	16E	10	15	45	0.80	16.7	0.0162
16	737-300	16A	NA	NA	37	1.03	17.2	0.0137
63	737-200	15C	5	8	45	0.95	17.9	0.0173
42	737-200	16C	16	30	56	1.26	19.5	0.0235
22	737-200	14D	NA	NA	60	1.74	21.5	0.0231
57	737-200	15C	14	18	39	1.08	23.3	0.0196
61	727-200	20C	54	128	71	1.26	24.2	0.0369
15	737-200	16D	NA	NA	55	2.18	24.4	0.0288
74	737-200	16C	18	24	47	1.83	32.7	0.0330
18	737-200	16D	NA	NA	13	0.85	40.2	0.0112

*Samples collected outside of boundary rows. *Concentration at actual conditions.

and maintained. The number of active smokers on a particular flight, and thus the number of cigarettes smoked, was not quantified because this would have disrupted airline operations.

Nicotine results in each table are reported in the manner recommended by the American Chemical Society (13). For results below the limit of detection, ND signifies none detected, and the detection limit is shown in parentheses. For results below the limit of quantitation, the measured quantity is given, and the limit of quantitation is presented in parentheses.

Included in Tables I and II are the results of one experiment performed to assess the spatial variability of nicotine concentrations on a single flight. Four concurrent measurements were performed during a 73-min flight. One sample (sample 1) was acquired at seat 3D in the forward portion of the no-smoking section, two samples (samples 2 and 3) were acquired at adjacent seats 19E and 19F in the no-smoking section on the boundary with the smoking section, and one sample (sample 4) was obtained at seat

24F in the smoking section. The observed nicotine concentrations (at actual conditions) were <0.03, 2.3, 1.9, and 42.2 $\mu\text{g}/\text{m}^3$, respectively. Twenty persons occupied the smoking section.

The results from this experiment show nicotine levels decreasing substantially from the smoking section to the no-smoking section. The smoker nearest seats 19E and 19F was seated one row distant on the opposite side of the aisle. The results suggest that nicotine (and therefore, ETS) concentration gradients may typically exist at boundary rows.

Bartlett's test for homogeneity of variances was used to test the nicotine concentration data. Test results supported a log-normal distribution. The concentration data were transformed to their logarithms in order to obtain homogeneity of variances and a normal distribution. The transformed data were then analyzed with a 3×2 factorial model ANOVA. Results indicate that the effect of aircraft type is not significant ($P = 0.1802$). On the other hand, the results show the effect of seating section (either

Table II. Results from Samples Collected in Smoking Sections of B727-200, B737-200, and B737-300 Aircraft

sample	aircraft type	seat	no. in smoking section	no. of cig smoked (mtld)	sampling time, min	nicotine		cig equiv
						µg	µg/m ³	
25	737-200	16E	NA	NA	50	ND (0.004)	ND (0.08)	NA
68	737-200	15C	13	26	60	ND (0.02)	ND (0.03)	NA
67	737-200	17C	20	37	55	0.04 (0.08)	0.6 (1)	NA
65	737-300	19E	22	37	50	0.04 (0.08)	0.7 (2)	NA
45	727-200	20E	25	88	105	0.04	0.4	0.0009
59	727-200	20B	21	50	72	0.05	0.7	0.0010
27	737-200	16D	NA	NA	55	0.16	2.1	0.0024
60	737-200	15B	NA	NA	45	0.11	2.3	0.0022
49	737-200	14C	10	17	82	0.19	3.1	0.0035
6	727-200	22F	NA	NA	179	0.98	4.5	0.0172
63	737-200	20C	24	30	25	0.23	8.6	0.0046
84	737-300	17B	10	17	50	0.46	8.8	0.0095
48	727-200	19B	10	23	70	0.75	10.2	0.0154
28	727-200	21B	17	32	57	0.82	10.5	0.0129
14	727-200	19D	NA	NA	60	1.07	11.0	0.0142
5	727-200	22B	NA	NA	91	1.66	14.9	0.0291
47	737-300	16C	35	123	105	2.05	18.7	0.0423
56	737-200	15C	11	6	16	0.42	22.1	0.0076
51	737-200	18E	7	11	45	1.44	30.2	0.0293
76	737-300	20E	15	19	37	2.01	39.5	0.0314
4	727-200	24F	20	48	72	3.07	42.2*	0.0653
78	737-300	23F	22	30	41	4.82	45.0	0.0397
62	737-200	17D	20	17	25	1.51	57.1	0.0307
79	737-300	22D	22	84	114	16.79	59.8	0.1466
52	737-300	16B	23	38	50	4.06	76.7	0.0825
43	737-200	16C	23	31	40	5.18	112.4	0.0967

* Concentration at actual conditions.

smoking or no smoking) to be significant ($P = 0.0477$) as well as the effect of interaction, namely, aircraft \times seating section ($P = 0.0766$). The model analyzed interaction with a type III sum of squares, which compensates for an unbalanced number of data and any interaction effect on the main effect terms.

The data strongly suggest that the significance of the difference in the nicotine concentrations between smoking and no-smoking sections would have been greater if samples had been collected more evenly in no-smoking sections. Only 9 of the 48 samples associated with no-smoking sections were collected outside of the boundary region; these nine samples tend to be associated with lower nicotine concentrations. The significance of the aircraft-seating section interaction is expected in view of the fact that the areas of smoking and no-smoking sections and ventilation characteristics differ among the three aircraft types.

The number of persons seated in the smoking section and the sampling time, when employed as covariates for the 3×2 factorial ANOVA model, were shown to be insignificant: $P > 0.5437$ and $P > 0.3221$, respectively. The absence of significance for the former is exemplified by the $0.4 \mu\text{g}/\text{m}^3$ result of sample 45 acquired in the smoking section of a B727-200 when occupied by 25 persons.

Table III summarizes the concentration results by aircraft type and seating section. Included in the table are data ranges and geometric means.

Mean nicotine levels in the aircraft investigated are substantially lower than mean levels observed in environments where the density of smokers is similar. For example, Muramatsu et al. (3) reported mean levels of 26.42, 38.73, and $47.71 \mu\text{g}/\text{m}^3$ in student cafeterias, conference rooms, and automobiles, respectively. The design of the aircraft's HVAC systems accounts for both the observed relatively low nicotine concentration levels and the absence of significant correlation with number of smokers.

Figure 2 shows the patterns of air circulation along the cross-section of a B727-200 aircraft. (Diagrams for the

Table III. Summary of Results from Sampling Nicotine in Aircraft

aircraft type	seating section	N	nicotine concn, $\mu\text{g}/\text{m}^3$	
			range	mean
727-200	NS	10	ND (0.03)-24.2	2.6
	S	8	0.4-42.2	6.8
737-200	NS	29	ND (0.04)-40.2	7.7
	S	11	ND (0.06)-112.4	45.5
737-300	NS	10	ND (0.4)-17.2	4.2
	S	7	0.7 (2)-76.7	21.5
total	NS	49	ND (0.03)-40.2	5.5
	S	26	ND (0.06)-112.4	9.2

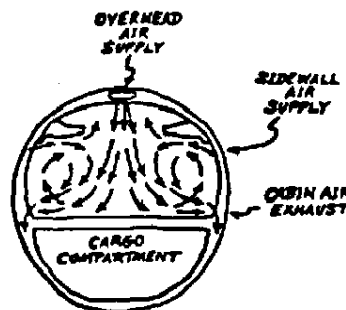


Figure 2. Schematic of airflow patterns for cross-section of B727-200 aircraft (17).

B737-200 and B737-300 aircraft are essentially the same.) Air supplies and exhausts are located in a manner that causes air to execute circular movements along a row of seats. Air enters the cabin from overhead vents and exits from vents located at foot level along cabin walls. Mirror-image circulation patterns distinguish port and starboard seats of each row. Air movement within a row also depends on the operation of overhead vents by passengers. Important aspects of the ventilation patterns shown in the

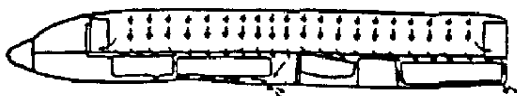


Figure 3. Schematic of airflow patterns along the length of B727-200 aircraft (17).

figures are that longitudinal movement of air in the cabins is suppressed, as is movement across the aisles. This longitudinal suppression of air movement is illustrated by Figure 3, showing ventilation patterns along the length of a B727-200's fuselage. (Diagrams for the B737-200 and B737-300 aircraft are essentially the same.) The high ventilation rates of the aircraft studied [for example, 26.5 air changes per hour for B727-200's, 22.7 air changes per hour for B737-200's, and 26.3 air changes per hour for B737-300's (10)] minimize the residence time of ETS in passenger cabins. Additionally, ETS will tend to remain within a single port or starboard row of seats owing to the effect of air movement patterns.

The effects of ventilation and air movement patterns and the relative isolation these effects impose on a port or starboard row of seats may account for those results where nicotine concentrations in smoking sections are below the limit of quantitation even though the sections are occupied by substantial numbers of passengers who presumably smoke. Similarly, the nicotine concentration results of this study, when viewed in light of the aircraft's ventilation characteristics, suggest that the port-starboard segregation approach utilized, for example, by European airlines, may be effective in reducing the exposure of persons seated in the no-smoking section to ETS.

The results of this study show that, to be adequate, models for air quality within aircraft cabins must account for the unique ventilation characteristics of aircraft. Models assuming the complete mixing of ETS in passenger cabins (14) are inappropriate for B727-200, B737-200, B737-300, and similar aircraft.

Most of the nicotine concentrations reported here have a high bias component due to the lack of barometric pressure data with which to adjust volumetric data from standard conditions to actual conditions. [Human respiration is not affected by the barometric pressures maintained in passenger cabins (15).] Barometric pressure data monitored on four sample runs indicate that concentration biases of up to 15% are possible.

The nicotine concentrations observed for this study are similar in magnitude to those reported by Muramatsu et al. (3). These workers, using a portable system attached to persons conducting the sampling, reported nicotine concentrations on Japanese domestic aircraft that ranged from 6.28 to 28.78 $\mu\text{g}/\text{m}^3$. The mean concentration of the seven samples was 15.18 $\mu\text{g}/\text{m}^3$. The authors concluded from these results that the exposure of persons to side-stream tobacco smoke, i.e., ETS, is very small. The authors, however, did not provide information regarding the types of aircraft, the sampling locations relative to the smoking sections, or the number of smokers; therefore, comparison with the results from the study reported here are limited.

Some researchers (3, 14, 16) have used the "cigarette equivalent" computational device to quantify exposure to ETS and thus to place such exposure in a convenient framework for discussion. Such computations assume an average daily breathing rate and an "equivalent cigarette" on the basis of the delivery of nicotine or "tar" in main-stream smoke. However, the term cigarette equivalent is inaccurate inasmuch as it suggests that persons thus ex-

posed are smoking, when in fact they are not. In addition, inhalation during smoking is deeper and more prolonged than during ordinary breathing, and breathing rates are variable rather than constant. Finally, the cigarette equivalent concept is highly manipulatable, because nicotine or tar deliveries vary over a wide range of values for different cigarette brands and because no single definition is currently recognized.

In spite of these shortcomings, the exposures represented by the nicotine levels observed for this study may perhaps be placed in perspective through use of the cigarette equivalent device. Accordingly, concentrations in no-smoking sections represent exposures ranging from 0.00004 to 0.037 cigarette equivalent per sampling period, with a geometric mean of 0.0041 cigarette equivalent per sampling period. Concentrations in smoking sections represent exposures ranging from 0.00008 to 0.15 cigarette equivalent per sampling period, with a geometric mean of 0.0082 cigarette equivalent per sampling period. These estimates in general indicate very low exposure relative to active smoking.

Conclusions

The results of this study show that (a) segregation significantly reduces the exposure of persons seated in no-smoking sections to ETS and (b) aircrafts' HVAC systems are primarily responsible for effecting this reduction. In addition, the results indicate that average exposures to ETS are orders of magnitude less than exposures represented by smoking a single cigarette.

Additional research is needed in order to define more precisely and completely the effect ETS has on air quality in passenger cabins of commercial aircraft. Future studies should be expanded to include measurements of other ETS constituents and to involve wide-bodied aircraft on longer flights.

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Registry No. Nicotine, 54-11-5.

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Oligomerization of 4-Chloroaniline by Oxidoreductases

Kathleen E. Simmons, Robert D. Minard, and Jean-Marc Bollag*

Department of Agronomy and Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802

■ Oxidation of aromatic amines by oxidoreductases can result in the formation of polyaromatic products. We incubated 4-chloroaniline with horseradish peroxidase and with a laccase from the fungus *Trametes versicolor*. Qualitative and quantitative analyses were performed on the oligomeric products. Both enzymes generated eight oligomers, which were isolated and identified. On the basis of their structures and rates of formation, a reaction scheme for the oxidative oligomerization of 4-chloroaniline was proposed. The scheme shows that, once the substrate was enzymatically oxidized, free-radical coupling followed, and three dimeric intermediates were produced. Each of the dimers initiated a nonenzymatic reaction pathway, and the combined pathways accounted for the formation of the first eight stable 4-chloroaniline-derived oligomers.

Introduction

Aniline-based herbicides readily decompose in the soil, but the resultant degradation products may be transformed into persistent xenobiotic species. Hydrolytic cleavage of the aliphatic portion of the herbicides produces substituted anilines, which often undergo oxidative polymerization and binding to soil organic matter. A study on the fate of substituted anilines in the soil found that at high concentrations (500 ppm) 40% of the applied material was recovered as polyaromatic products and 50% was bound to soil organic matter (1). At low concentrations (1.25 ppm), 90% of an aniline soil residue was bound to soil constituents with only trace amounts recovered as extractable oligomers (2). It is likely that the processes leading to polymerization are also responsible for incorporation of the anilines into humic substances.

Models of oxidative reactions are essential for understanding the transformation of substituted anilines in soil. Numerous studies have been conducted on the one-electron oxidation of anilines using oxidoreductases, such as horseradish peroxidase and the laccases of *Trametes versicolor* and *Rhizoctonia praticola* (3-7). Some of the aniline-derived oligomers were structurally determined, but neither comprehensive product identifications nor quantitative analyses were reported.

In a previous work, we identified the structures of all products formed in the oxidoreductase-initiated polymerization of 4-chloroaniline and developed a method for substrate and product quantitation (8). In this investigation, we apply the quantitative method to follow 4-chloroaniline disappearance and product formations as a function of enzyme incubation times. We compare the product profiles resulting from the reactions catalyzed by horseradish peroxidase and the laccase of *T. versicolor*. On

the basis of product structures and their relative amounts, we postulate reaction pathways for the oxidative polymerization of substituted anilines in general and for 4-chloroaniline in particular.

Materials and Methods

Chemicals. 4-Chloroaniline was purchased from Aldrich Chemical Co. (Milwaukee, WI) and was 98+ % pure as confirmed by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC).

Enzyme Assays. Horseradish peroxidase with an RZ (Reinheitzahl) of 0.43 and an activity of 45 purpurogallin units/mg of solid was purchased from Sigma Chemical Co. (St. Louis, MO). A purpurogallin unit is defined as the amount of enzyme that forms 1.0 mg of purpurogallin from pyrogallol in 20 s at pH 6.0 and 20 °C. The absorbance change is measured at 420 nm.

The extracellular laccase of *T. versicolor* was isolated from growth media and purified as previously described (7). Laccase activity is given in DMP (2,6-dimethoxyphenol) units. A DMP unit is defined as the amount of enzyme causing a change in absorbance at 468 nm of 1.0 unit min⁻¹ at pH 4.2 of a 3.5-mL sample containing 3.24 μmol of 2,6-dimethoxyphenol. Absorbance was measured with a Model 2000 spectrophotometer (Bausch and Lomb, Inc., Rochester, NY).

Unless otherwise specified, enzyme assays were conducted in citrate-phosphate buffers (pH 4.2) with 1 μmol/mL 4-chloroaniline at 25 °C. Horseradish peroxidase assays contained 2.5 μmol/mL hydrogen peroxide and 0.012 purpurogallin unit/mL enzyme; 20 DMP units/mL was used in the laccase assays. Boiled enzymes served as controls.

High-Performance Liquid Chromatography. At the specified times (0-120 min), enzyme activity was halted by the addition of an equal volume of acetonitrile to a 2.5-mL aliquot of the assay solution. The 5.0-mL sample was then passed through a 0.2-μm pore Nylon 66 filter (Schleicher & Schuell, Keene, NH), and 175 μL was immediately analyzed by HPLC. All quantitative data points represent the average value of triplicate sample injections.

Analysis was performed on a Waters Associates (Milford, MA) high-performance liquid chromatograph. The system consisted of a U6K injector, M45 and 6000 pumps run by a Model 720 System Controller, a Lambda Max 450 LC spectrophotometer set at 280 nm (0.05 AUFS), and a Model 730 Data Module.

Reverse-phase separation was performed on a 15 cm × 4.6 mm Supelcosil LC18 (octadecylsilica) column of 5-μm particle size (Supelco, Inc., Bellefonte, PA). The mobile phase at a flow rate of 1.5 mL/min consisted of an aqueous